

### REMARKS

Reconsideration and allowance are respectfully requested.

Claims 34-42 and 46 are now pending, with Claim 34 being the sole independent claim. Claims 43-45 have been cancelled without prejudice to or disclaimer of the subject matter recited therein. The specification has been amended to delete references to internet hyperlinks.

Turning now to the Office Action mailed August 26, 2002:

Claims 34-36, 39-42, and 46 have been rejected under 35 U.S.C. §112, first paragraph as allegedly being non-enabled.

Applicants respectfully traverse this rejection..

Applicants submit that the specification, when combined with knowledge readily within the level of one of ordinary skill at the time of filing, is sufficient to enable one of ordinary skill to make and use the invention as claimed without undue experimentation.

An IDS filed simultaneously herewith includes two journal articles, which Applicants believe are exemplary of the knowledge of one of ordinary skill in the art as of the priority date of the instant application:

Carpenter et al., "Structure of dehydroquinase synthase reveals an active site capable of multistep catalysis," Nature July 16, 1998, 394:299-304.

Bischoff et al., "Cloning of cDNA encoding a 3-dehydroquinase synthase from a higher plant, and analysis of the organ-specific and elicitor-induced expression of the corresponding gene," Plant Mol. Biol. 1996 Apr. 31(1): 69-76.

Carpenter et al. identified nine key amino acids of an *Aspergillus nidulans* dehydroquinase synthase involved in critical interactions in its proposed catalytic mechanism: Arg130, Lys152, Asn162, Glu194, Asn268, His271, His275, His287, and Lys356. See Nature 394:299-304, 299-300, Figs. 1-3. These were determined using 1.8 Å resolution crystallography studies.

The nine amino acids identified by Carpenter et al. are conserved in SEQ ID NO:6 of the instant claims, as shown by black highlighted boxes in the sequence alignment on Appendix A, attached hereto.

These nine amino acids are also conserved in the following additional dehydroquinase synthase sequences shown on Appendix A: SEQ ID NO:2 (corn), SEQ ID NO:4 (rice), and SEQ ID NO:8 (partial from wheat) from the present application; *E. coli* (NCBI General Identifier No. 114181); *L. esculentum* (tomato); *B. subtilis*; *M. tuberculosis*; *P. carinii*; and *S. cerevisiae*.

Additionally, Appendix A shows amino acids conserved among all sequences, as indicated by an asterisk (\*) above the alignment, as well as amino acids conserved among the plant sequences only, as indicated by a plus (+) above the alignment.

Appendix B, also attached hereto, contains a chart showing percent sequence identities (upper triangular half) and sequence divergences (lower triangular half), calculated using the Clustal V method of alignment with default parameters, for any given pair of amino acid sequences disclosed on Appendix A.

Finally, Bischoff et al. demonstrated that an *L. esculentum* (tomato) dehydroquinase synthase (NCBI General Identifier No.18654278) was capable of supplementing a strain of *E. coli* that is deficient in such an enzyme, even though, as shown on Appendix B, this *L. esculentum* sequence is 52.2% identical to an *E. Coli* sequence (NCBI General Identifier No. 114181).

Applicants submit that one of ordinary skill in the art, having knowledge of the foregoing and upon reading the specification, would be enabled to make and use the claimed invention without undue experimentation.

Accordingly, withdrawal of the 35 U.S.C. §112, first paragraph rejection of Claims 34-36, 39-42, and 46 is respectfully requested.

Applicants believe the foregoing to be responsive to each of the points raised in the Office Action. Early and favorable notification of allowance is hereby solicited.

Applicants' undersigned may be reached at the below-listed numbers.

Respectfully submitted,



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Dated: 2-26-03

Attachments: Appendices A and B

PATENT

# APPENDIX A

Alignment of the dehydroquinase synthases from *E. coli* (NCBI gi No.114181), corn contig assembled of clones cbn2.pk0047.e10 and cca.pk0019.e12 (SEQ ID NO:2), rice clone rls72.pk0035.a10 (SEQ ID NO:4), soybean clone se3.pk0029.f9 (SEQ ID NO:6), wheat clone wr1.pk0011.d5 (SEQ ID NO:8), *L. esculentum* (NCBI gi No.18654278), *A. nidulans* (amino acids 1-384 from NCBI gi No.6226554), *B. subtilis* (NCBI gi No.399057), *M. tuberculosis* (NCBI gi No.3915615), *P. carinii* (amino acids 1-383 from NCBI gi No.2492977), and *S. cerevisiae* (amino acids 1-392 from NCBI gi No.114166). The program uses dashes to maximize the alignment. The amino acid number is indicated at the left of each sequence.

1	-----	-----	-----	<i>E. coli</i>
1	MAASASSLLAAPASSCGAISPQLPRGAP	---AASVASPSRHS CYL-LRASPSRRHRS	SEQ ID NO:2	
1	MAAAASSSSLLAAASSSSRAAASARRAPSASPA	AAAASLPSPRASCAPPLRASAAATLRS	SEQ ID NO:4	
1	MAST-ATNFSLSLCANQOTPIPKPSFFSNHNLHFN	SNWAWA-----SVSTSRKS-	SEQ ID NO:6	
1	-----	-----	SEQ ID NO:8	
1	MASSFCPKQALSFTNSTH-QLHQSR	AIPRDIHVRFPAPVSSPSSRC--GLKSKATTRLK-	<i>L. esculentum</i>	
1	-----	-----	<i>A. nidulans</i>	
1	-----	-----	<i>B. subtilis</i>	
1	-----	-----	<i>M. tuberculosis</i>	
1	-----	-----	<i>P. carinii</i>	
1	-----	-----	<i>S. cerevisiae</i>	
	+	+	++++	++++
1	-----	-----	-----	<i>E. coli</i>
56	RFVANAAPTMQPPA	---ESRVSTVVDVLDGDRSYPIYIGAGLLDEP	-DLLQRHVHG-KRV	SEQ ID NO:2
61	RVVASAAPAMQPPP	---ASRVSTVVDVLDGDRSYPIYIGAGLLDEP	-DLLQRHVHG-KRV	SEQ ID NO:4
52	RICATSSQVMDPSAAKSEPALPTIVEVDL	GSRSYPIYIGSGLLNQP-DYLQRHVHG-KRV	SEQ ID NO:6	
1	-----	-----	-----	SEQ ID NO:8
57	VLATSATKVMDSHSSSKASSQAPTVEVDL	GTRSYPIYIGAGLLDQP-DLLQRHIHG-KRV	<i>L. esculentum</i>	
1	-----	-----	-----	<i>A. nidulans</i>
1	-----	-----	-----	<i>B. subtilis</i>
1	-----	-----	-----	<i>M. tuberculosis</i>
1	-----	-----	-----	<i>P. carinii</i>
1	-----	-----	-----	<i>S. cerevisiae</i>

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+++++ ++++++ + +++ + ++++++ ++++++ * + ++++++ ++++++
38 MLVTNETLAPLYLDKVRGVLEQAG--VNVDSVIL---PDGEQYKSLAVLDTVFTALLQK E. coli
111 LVVTNTTVAPLYLDKVTWALTHNNLNVSVESVIL---PDGEKYKNMDTLMKVFDKAVES SEQ ID NO:2
116 LVVTNTTVAPLYLEKVTWALTHNNPNVSVESVIL---PDGEKYKDMGTLMKVFDKAVES SEQ ID NO:4
110 LVVTNETVAPLYLDKVVDALTRGNPNVSVESVIL---PDGEQYKDMDTLMKVFDKAIES SEQ ID NO:6
1 -----
115 LVVTNTTVAPLYLDKVTISALTDGNPNVTVESVIL---PDGEQFKNMETLMKVFDKAIES L. esculentum
40 VLVTDTNIGSIYTPSFEEAFRKRAAEITPSRLLIYNRPPEVSKSRQTKADIEDWMLSQ A. nidulans
40 MFVTDEEVDRLYGDEMLHLLQEKWP---VKKVTV---PSGEQAKSMDMYTKLQSEAIRF B. subtilis
41 AVVHQPLAET-ABEIRKRLAGK--VDAHRIE-----PDAEAGKDLPVVGFIWEVLGRI M. tuberculosis
39 VIITDSNIETLYIPSFKTYFISMAKQRSINSRLLFFTIIPGEKSKSRKTKALIEDWLLSE P. carinii
39 VICNDTNLSKV--PYQQLVLEFKASLPESGRLTTYVVKPGETSKSRETKAQLEDYLLVE S. cerevisiae

+++++ ++++++ ++++++ ++++++ ++++++ ++++++ ++++++ ++++++ ++++++
92 PH--GRDTTLVALGGGVVGDLTGFAAASYQ R GVRFIQVPTTLLSQVDSSVGG K TAVNHPL E. coli
167 RF--DRRCTFVALGGGVIGDMCGFAAAAF R GVNFIQIPTTLLMAQVDSSVGG K TGINHPL SEQ ID NO:2
172 RL--DRRCTFVALGGGVIGDMCGFAAAAF R GVNFIQIPTTLLMAQVDSSVGG K TGINHPL SEQ ID NO:4
166 RL--DRRCTFVALGGGVIGDMCGFAAASAF R GVNFIQIPTTVMAQVDSSVGG K TGINHRL SEQ ID NO:6
1 -----ARAFVALGGGVIGDMCGFAAAAF R GVNFIQIPTTLLMAQVDSSVGG K TGINHPL SEQ ID NO:8
171 RL--DRRCTFVALGGGVIGDMCGYAAASYL R GVNFIQIPTTVMAQVDSSVGG K TGINHPL I. esculentum
100 NPPCGRDTVIALGGGVIGDLTGFAVASTYM R GVRVYQVPTTLLAMVDSSIGG K TAIDTPL A. nidulans
93 HM--DRSSCIAFGGVVGDLAGFVAATEM R GIDFIQMPPTLLAH-DSAVGG K VAVNHPL B. subtilis
94 GI--GRKDALVSLGGAATDVAGFAAATWL R GVSIVHLPPTLLGMVDAAVGG K TGINTDA M. tuberculosis
99 K--CTRDTVIIAIGGVIGDLVGYSVATEM R GVRFIQIPTTLLAMVDSSIGG K NSINTSY F. carinii
97 G--CTRDTVMAIGGVIGDMIGFVASTEM R GVRVYQVPTSLAMVDSSIGG K TAIDTPL S. cerevisiae

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**	*	+ *	+ +	+ *++	+ + +	+ + + +	+ + + + +	+ + + + + +	+ + + + + + +	+ + + + + + + +	+ + + + + + + + +	+ + + + + + + + + +	+ + + + + + + + + + +
150	GK	N	MIGAFYQPASVVVDLCLKTLPFRELASGLA	E	VIKYGIIIDGAFFNWLEENLDALLR	E.	coli						
225	GK	N	LIGAFYQPQCVLIDTNTLTLPDRELASGIA	E	VVK'iGLIFDAFFFEWQEKNMPKLLA	SEQ ID NO:2							
230	GK	N	LIGAFYHPQCVLIDTETLTLPDPELASGIA	E	VVF'YGLIRDAFFFEWQEKNMPALLA	SEQ ID NO:4							
224	GK	N	MIGTFYQPQCVLIDTDTLTLPDRELASGLA	E	VIKYGLIRDAEFFEWEKNNMHLLLA	SEQ ID NO:6							
55	GK	N	LIGAFYQPQCVLIDTETLTLPDRELASGVA	E	VVKYGLIRDAFFFEWQEKNMAAILA	SEQ ID NO:8							
229	GK	N	MIGAFYQPQCVLIDTDLTLTPDRELASGLA	E	VIKYGLIRDAEFFEWEQNMPLLLLA	L. esculentum							
160	GK	N	LIGAIWQPKIYIDLEFLETLPVREFINGMA	E	VIKTA AISSEEEFTALEENAE -- - T	A. nidulans							
150	GK	N	LIGAFYQPKAVLYDTDFLRSPLPEKELRSGMA	E	VIKHAFIYDRAFL E-ELLNIHSLRD	B. subtilis							
152	GK	N	LVGAFHQPLAVLVDLATLQTLPDEMIGCMA	E	VVKAGFIADPVIDLIEADPQAALD	M tuberculosis							
157	GK	N	AIGTIWQPERIFIDFTFLETLTLEKEFINGIA	E	LIKTTIIIWDESEFALENISE -- - K	P. carinii							
155	GK	N	FIGA F W Q P K F V L D I K W L E T L A K R E F I N G M A	E	VIKTACIWNAD E FTRLESNASLFLN	S. cerevisiae							

[illegible]

243	L	N	LG	H	TFG	H	TFG	H	AIEAEMGYGNWL	H	GEAVAAGVMVMAARTSERLQFS-SAETQRIITLLKRAG	E. coli
318	L	N	LG	H	TFG	H	TFG	H	AIETGTGYGAWL	H	GEAVAAGTMATDMSHRLGWID-DSIRKRVVDILKQAK	SEQ ID NO:2
323	L	N	LG	H	TFG	H	TFG	H	AIETGTGYGAWL	H	GEAVAAGTMADMSHRLGWID-ESIKKRAIDILEKAK	SEQ ID NO:4
317	L	N	LG	H	TFG	H	TFG	H	AIETGVGYGQWL	H	GEAVAAGTMADMSYRLGWID-DSLVRVGDILKQAK	SEQ ID NO:6
148	L	N	LG	H	TFG	H	TFG	H	AIETGLGYGEWL	H	GEAVAAGTMADMSYRLGWID-ESIKKRTFDILDQAK	SEQ ID NO:8
322	L	N	LG	H	TFG	H	TFG	H	AVETGVGYGQWL	H	GEAVAAGTMADMSRRRLGWID-DSLVRVQKILQQA	L. esculentum
267	L	N	WG	H	SIG	H	SIG	H	AIEAILT-PQIL	H	GECVAIGMVKEAELARHLGILK-GVAVSRIVKCLAAAYG	A. nidulans
242	L	N	FG	H	TLG	H	TLG	H	AVEAEYGYGQIT	H	GDAVALGMQFALYISEKT--VGCENDRKRLVSWLKSLG	B. subtilis
245	L	N	YG	H	TLG	H	TLG	H	AIERRERY-RWR	H	GAAVSGLVFAAELARLAGRLD-DATAQRHRTILSSIG	M. tuberculosis
266	L	N	FG	H	SIG	H	SIG	H	AIETVLA-PYIL	H	GESISIGMVKEAELSRHLGILN-PNVVSRLIKCLNTWG	P. carinii
275	L	N	FG	H	SIG	H	SIG	H	AYEAILT-PQAL	H	GECVSIGMVKEAELSRYFGILS-PTQVARLSKILVAYG	S. cerevisiae

  

302	LPVN-G	-----PREMSAQAYLPHMLRD	K	KVLAGEMLRIL-PLAIGKS-----EVR	E. coli
377	LPIA-P	-----PETMTVEKFKNIMAVD	K	KVADGLLRLILILKGPLGCC-----VFT	SEQ ID NO:2
382	LPIT-P	-----PEAMTVEKFKSIMAVD	K	KVADGLLRLILILKGPLGSC-----VFT	SEQ ID NO:4
376	LPTA-P	-----PETVTVDMEKSVMAVD	K	KVADGLLRLILILKGPLGNC-----VFT	SEQ ID NO:6
207	LPVT-S	-----PKGMTVEKFRNIMAVD	K	KVADGLLRLILILKGPLGCC-----VFT	SEQ ID NO:8
381	LPTS-P	-----PETMTVEMFKSIMAVD	K	KVADGKLRLILILKGLGNC-----VFT	L. esculentum
325	LPTSLKDARIRKLTAGKHCSVDQLMFNMALD	K	KNDGPKKKIVLLS-AIGTPYETRASVVA	A. nidulans	
300	YPSQ-I	-----RKETETSVLLNRMMND	K	KTRGGKIQFIVLNE-LGK-----VAD	B. subtilis
303	LPVSYD	-----PDAL--PQLLEIMAGD	K	KTRAGVLRFFVLDG-LAKP-----GRM	M. tuberculosis
324	LPTSFKDRRFKEVILGKKHLIEDILEIMSID	K	KNDSNNKKIVLLS-AIGKTYEKKASSVS	P. carinii	
333	LPVSPDEKWFKELT	LHKKTPLDILKKMSID	K	KNEGSKKKVVILE-SIGKCYGDSAQFVS	S. cerevisiae

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+ +  
345 SGVSHLVNLNAIADCQ-----SA  
421 GDYDGNALDETLHAF-----DN  
426 GDYC-----RSTC-----R  
420 GDYDRKALDNTLRAF-----KS  
251 GEYDRKALDETLRAF-----DN  
425 GDYDQKALDETLRAFS-----KS  
384 NEDIRVVVLAPSIIEVH--PGVAHSSN  
342 HTFSRNELESWLNKWRLE----ETS  
345 VGPDPGLLVTAYAGVC-----AP  
383 DDDIRTILSQNILLYGIPLNAFQKH  
392 DEDLRFILTDETLVYPFKDIPADQQ

*E. coli*  
SEQ ID NO:2  
SEQ ID NO:4  
SEQ ID NO:6  
SEQ ID NO:8  
*L. esculentum*  
*A. nidulans*  
*B. subtilis*  
*M. M tuberculosis*  
*P. carinii*  
*S. cerevisiae*



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# APPENDIX B

	E. coli	SEQ ID NO:2	SEQ ID NO:4	SEQ ID NO:6	SEQ ID NO:8	L. esculentum	A. nidulans	B. subtilis	M. tuberculosis	P. carinii	S. cerevisiae	
E. coli	***	52.5	52.5	52.8	55.2	52.2	33.1	35.9	35.6	29.0	32.0	E. coli
SEQ ID NO:2	72.1	***	84.4	73.2	88.8	72.4	26.4	35.1	34.8	25.3	28.1	SEQ ID NO:2
SEQ ID NO:4	69.6	14.3	***	70.2	85.8	69.5	26.8	34.0	35.1	25.3	27.4	SEQ ID NO:4
SEQ ID NO:6	70.8	31.3	35.6	***	82.5	75.7	27.1	34.5	32.0	25.6	27.4	SEQ ID NO:6
SEQ ID NO:8	68.2	11.7	11.6	20.0	***	81.0	38.1	38.8	41.4	33.6	36.6	SEQ ID NO:8
L. esculentum	73.5	32.7	36.2	28.1	22.0	***	26.8	34.5	32.3	26.3	28.4	L. esculentum
A. nidulans	111.9	132.5	131.9	131.3	101.6	126.2	***	26.0	29.3	50.7	48.5	A. nidulans
B. subtilis	111.0	104.7	110.0	106.7	95.4	107.7	138.4	***	28.2	27.3	28.7	B. subtilis
M. tuberculosis	111.7	111.5	107.5	121.0	94.1	120.3	148.1	131.0	***	26.2	30.1	M. tuberculosis
P. carinii	129.6	140.4	137.0	140.4	116.2	136.2	69.6	142.9	157.8	***	47.4	P. carinii
S. cerevisiae	127.4	146.9	144.9	140.9	106.5	138.0	75.6	137.4	145.5	85.3	***	S. cerevisiae
	E. coli	SEQ ID NO:2	SEQ ID NO:4	SEQ ID NO:6	SEQ ID NO:8	L. esculentum	A. nidulans	B. subtilis	M. tuberculosis	P. carinii	S. cerevisiae	